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Effect of aqueous extract of *Artemisia herba-alba* on functional sperm parameters of male rats

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Abstract

Artemisia species have a high economic value in several fields, as food plants and as antihelminthic and antimalarial in medicine Many of the species of Artemisia have found their way into folklore medicine. In Libya commonly referred to as wormwood also called Alshih, the genus Artemisia is represented in Libya by seven species namely *A. arborescens L, A. campestris L, A. glutinosa, A. herba-alba, A. judaica, A. monosperma* and *A. variabilis*. This study, for the first time, documented the effects of an aqueous extraction of Artemisia Herba-Alba in the rat investigating male reproductive functions. From the results obtained, it is evident that the herbal extract in the low dose (10mg/kg) had a beneficial effect on all parameters, with the exclusion of sperm morphology. In this regard, active compounds present in the plant might have caused an increased production of serum testosterone which resulted in the enhancement of sperm quality in the rat. Another explanation for the increase in sperm functionality might have been induced by the presence of components within the plant of *Artemisia Herba-Alba* causing the efficient removal of zinc from spermatozoa enabling them to reach optimal motility. It is also possible that these two explanations given above could have occurred simultaneously, thereby enhancing the positive effects observed within this study. This study indicated that the traditional use of this herbs has effects on male reproductive health.

Keywords: *Artemisia Herba-Alba* , Sperm motility, Sperm Morphology, Sperm ROS production, Mitochondrial Membrane Potential (Δψm), DNA fragmentation , Testosterone Concentration

Introduction

In many ancient civilizations, plants were used to treat various diseases. Recently, folk medicines are considered an important step due to multidrug resistance and other factors.

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Plants are a source of natural remedies, biologically active compounds or extracts to provide new and innovative products for the treatment and prevention of diseases are formidable. Such drugs are a new target for researchers to find a cure for diseases, including various cancers. [1]. Listed Artemisia in the flora of Libya, he recorded 5 species are reported from Libya [2]. Several species of Artemisia have found their way into folk medicine and have been used as a medicinal herb. In Libya, the genus Artemisia is represented by seven species [2]. They are A. arborescens L, A. campestris L, A. glutinosa, A. herba-alba, A. judaica, A. monosperma and A. variabilis. It is known that traditional healers make use of herbs to treat a variety of diseases as they rely on symptomatic diagnoses and prognosis of these diseases [2]. However, the possible adverse effects of various medicinal plants on fertility remain an enigma. In this regard, it is important to note that some plants contain estrogenic substances which may induce alterations to gametogenesis production [3]. Several authors have been investigating a variety of medicinal plants including Morinda lucida, Ricinus communis and Yohimbe and proved that these plants could potentially cause reductions in sperm density alter androgenic secretion [4]. and reduce motility and density of mice spermatozoa Conclusive studies by [5]. on Hypericum perforatum commonly used to treat mental disorders and nerve pain, have shown that the herb has a detrimental effect on some sperm functions like motility, sperm oolemma binding and DNA integrity. Scientists consider fertilization to be a very complex process that involves a chain of events. Therefore, one needs to take into account that a large amount of factors require thorough investigation. Also, the contribution of each sperm parameter weight to the multifactorial process that is fertilization [6]. It remains a mystery. In this regard, sperm quality is definitely an important factor in determining successful fertilization [7]. Thus, the process of increasing the production of an adequate amount of mature, motile and functionally competent sperm is of paramount importance for the male to be able to procreate [8]. For this reason, traditional semen analysis is used in order to determine the quality of sperm within a semen sample. Sperm concentration kinetics [9,10]. Marked morphology are some of the parameters that can be evaluated in an attempt to indicate the probability of successful fertilization. [11,12]. Even though the conventional semen analysis remains the cornerstone within andrological laboratories, the determination of other perhaps more sensitive parameters is worth looking into. However, the evaluation of parameters aside from the conventional parameters are often more expensive and time-consuming and not always available in routine laboratories. Also, authors like [13,14]. have reported that a number of men showing normal parameters after standard semen analysis, remain unable to conceive. Earlier reports also documented on the inadequacy of a semen analysis to predict fertilization outcome [15]. Taking in consideration that defective sperm function has been linked to loss of fertilization in males [16]. the reasons for sperm malfunctioning require extensive examining. In this regard, parameters investigated within this particular animal study include motility, sperm morphology, sperm mitochondrial membrane potential ($\Delta\psi m$), sperm reactive oxygen species (ROS) production, sperm DNA fragmentation and testosterone concentration [17].

Aim of the study

The effect of medicinal plants in terms of male reproduction is a research area left unexplored. For this reason, an in vivo study was conducted in an attempt to explicate the

effect of an aqueous extraction of *Artemisia Herba-Alba* in the rat. In addition to functional sperm parameters, this study compared body weight, organ weight and serum testosterone concentration in treated rats versus the control group. The current study is indeed a follow up investigation on an in vitro study conducted by [18]. In which the effects of the herbal extract were tested on human ejaculated semen samples. Results from the in vitro study suggested that *Artemisia Herba-Alba* extract had a detrimental effect of sperm function, this being in total contrast to claims made by traditional healers that the plant has fertility enhancing abilities. In an attempt to either disproof or confirm anecdotal claims made by traditional healers, the present study was performed.

MATERIALS AND METHODS:

1. Animals used .

Thirty adult male Sprague–Dawley rats weighing between 250-300 grams were kept in standard cages within the animal facilities of the Department of Medical Bioscience with temperature ranging between 20°C and 23°C. Animals were fed with standardized rat feed and water ad libitum and standard laboratory conditions 12h light and 12h dark were maintained.

2. Collection and preparation of plant material.

Artemisia Herba-Alba was collected from Coastal area (Al-Hishaa ,20 Km) is located south of Derna City. The plant species was identified and authenticated by A. Elmogasapi, a Botanist of the Department of Botany at university of Benghazi. The plant were thoroughly washed, cut into smaller pieces and allowed to dry in a drying oven at 25°C. Afterwards, an aqueous extract was prepared by infusing the plant in boiled distilled water for 4 hours. Subsequently, the infusion was filtered using a water filtration pump to ensure thorough separation of herbal material from distilled water. The remaining crude herbal material was discarded and the filtered infusion was freeze-dried into a fine powder using the Alpha HED 10 freeze-dryer (Christ Freeze dryers, Osterode am Harz, Germany). The freeze-drying process took between two to three weeks depending on the volume of *Artemisia Herba-Alba* infusion being freeze-dried. The powdered herbal extraction was then stored at 4ºC until further use.

3. Force feeding and sacrificing of rats.

Rats were segregated into control and experimental groups: (n=10 per group) **"Control"** (group of rats force fed with normal tap water for 52 days) **"Low dose"** (group of rats that were force fed with10mg/kg *Artemisia Herba-Alba* extract for 52 days) **"High dose"** (group of rats that were force fed with 100mg/kg *Artemisia Herba-Alba* extract for 52 days), Body weights of rats were recorded daily and the overall well being of the animals were monitored throughout the feeding process. At the end of the feeding period (52 days), animals were sacrificed by means of rapid cervical dislocation. Immediately after sacrificing, blood was aspirated directly from the heart for the determination of serum testosterone concentration which was performed at a later stage. Blood was incubated for one hour at 37°C and then centrifuged until the serum portion was separated from the pellet. Serum was collected in Eppendorf tubes (Greiner Bio-One GmbH, Frickenhausen,

Germany) and kept in the -20°C freezer until further use. Afterwards the testes, epididymis, seminal/coagulating glands, kidneys and the liver were removed and the respective organ weights were recorded. Spermatozoa were isolated from the caput- and cauda epididymides for functional analysis of sperm parameters.

4. Sperm collection and determination of motility.

The epididymis was placed on a warmed up Petri dish in 3ml of HTFM-HSA medium. Human Tubular Fluid Medium (HTFM) was made up according to [19]. The ionic composition of the medium was as follows; 2.9655g/ml NaCl (Merck, Modderfontein, South Africa), 0.175g/ml KCl (Merck), 0.1505g/ml $CaCl_2XH_2O$ (Merck), 0.025g/ml $MgSO_4X7$ H_2O (KIMIX, Eppindust, South Africa), 0.025g/ml KH_2 PO_4 (Merck, 0.00025g/ml Phenolred, 1.05g/ml $NaHCO_3$, 0.25g/ml Glucose (anhydrous), 0.018g/ml Na-Pyruvate (VWR International Ltd. Biochemical, Pool, England), 1.9991ml Na-Lactate (60% Syrup), 2.603g/ml HEPES (Sigma Aldrich, Steinheim, Germany).

5. Detection of Sperm ROS production.

For the determination excessive sperm reactive oxygen species (ROS) production the protocol according to [19] was followed. In brief, ROS production was determined by using the fluorescing probe, dihydroethidine (DHE) (Molecular Probes, Eugene, USA). DHE is enzymatically dehydrogenated to ethidium that intercalates with DNA and can be visualized as sperm that produced excessive ROS.

6. Determination of Mitochondrial Membrane Potential (Δψm).

Any disruption to the mitochondrial membrane potential (Δψm) will lead to the alteration of mitochondrial functionality. In this regard, intact or disturbed Δψm was determined by means of the DePsipher stain (DePsipher kit, catalogue number: TA700; R&D Systems, Abingdon, UK). The protocol provided by the manufacturer was modified as follows: A 1:10 dilution of Reaction Buffer was prepared in distilled water and pre-warmed at 37°C before use. Following this, stabilizer provided within the kit was added (20µl of stabilizer was added to every 1ml of prepared buffer).

7. Determination of Sperm Morphology.

Smears were made after pipetting 20µl of sperm sample onto slides. After allowing these smears to air dry, they were fixed by immerging them in methanol (Sigma Aldrich, Steinheim, Germany) at 4°C for 25 minutes. In order to determine sperm morphology a modified Papanicolaou staining technique was employed. Staining procedure followed by submerging slides (for at least 10 times per solution) in the following; 80% ethanol, 70% ethanol, 50% ethanol and then in distilled water. Following this, slides were then put in Harris Haematoxylin, Papanicolaou solution 1a (Merck 9253) (Merck and Co., Inc. Whitehouse Station, USA) for 15 minutes and then rinsed with running water for 3-5 minutes. Next, slides were submerged in 0.5% HCl; this particular submerging step was repeated after which slides were rinsed with running water for about 5 minutes. Subsequent to the rinsing step, slides were then placed in Scott's water (20g $MgSO_2X7H_2O$ and 2g $NaHCO_3$ dissolved in 1000ml distilled water).

8. Determination of DNA fragmentation by means of the TUNEL assay.

The TUNEL (terminal deoxynucleotidye transferase mediated dUTP nick-end labeling) assay is used for the detection of DNA strand breaks [20]. Adhering to the protocol provided within the kit (Detection System Fluorescein, Promega, Mannheim, Germany) and as described previously by [21]. In this study, the percentage of green fluorescing sperm (TUNEL-positive sperm) was determined. Also, slides without TdT enzyme were prepared which served as negative controls.

9. Determining Serum Testosterone Concentration.

For the determination of serum testosterone concentration the Testosterone ELISA kit (DRG Instruments GmbH, Marburg, Germany) was used. Twenty five µl of the standards were plated into the wells of the first row of the 96-well plate which was provided with the kit. Thereafter, 25µl of the respective 30 samples were added to previously assigned wells. Next, 200µl of conjugate enzyme were added to wells with standards and samples. The plate was then placed on a stirrer for an hour at room temperature. During this incubation step, a washing buffer was prepared by adding 12.5 ml of buffer to 500 ml distilled water. After incubation, wells were thoroughly submerged and washed with washing buffer. Following this very crucial washing step, the plate was tapped dry on tissue paper. Next, 200 µl of warmed up substrate were added to the wells. The plate was then incubated for 15 minutes in total darkness to allow for colour change to occur. Then, a 100µl of stop solution was added, after which plate was inserted into the Spectrophotometer (Multiskan, Thermo Fisher Scientific Inc, Waltham, US) and a reading was taken at a wavelength of 480nm.

10. Statistical analysis.

Statistical analysis was performed using the MedCalc software (Version 9.3.2.0; Mariakerke, Belgium). The Kolmogorov-Smirnonov test was applied to determine normal distribution. As all the parameters in this particular study showed normal distribution, for further statistical evaluation parametric tests like Pearson correlations as well as independent student t-tests were performed. A P-value of $P<0.05$ was considered to be significant. If not mentioned otherwise, data are expressed as mean±SD.

Results:

1. Effect of an aqueous extraction of *Artemisia Herba-Alba* on various parameters.

1.1 Sperm motility

As mentioned before, motility is the most obvious sperm function required for successful fertilization. Therefore, it is of paramount importance that the effect of treatment with *Artemisia Herba-Alba* extract on sperm motility is elaborated on in greater detail. Sperm motility after treatment with the low dose (10mg/kg) of *Artemisia Herba-Alba* extract was (57.08±7.39%) significantly higher (P=0.042) than that within the control (47.13±12.90%) (Figure 1). Although the high dose (100mg/kg) of *Artemisia Herba-Alba* extract also resulted in an increase in sperm motility (50.18±8.45%), this difference to the control (47.13±12%) did not show any significance (P=0.5737), instead, compared to the low dose, a decrease could be noticed, which did not reach significance level (P=0.0678). (Figure 1).

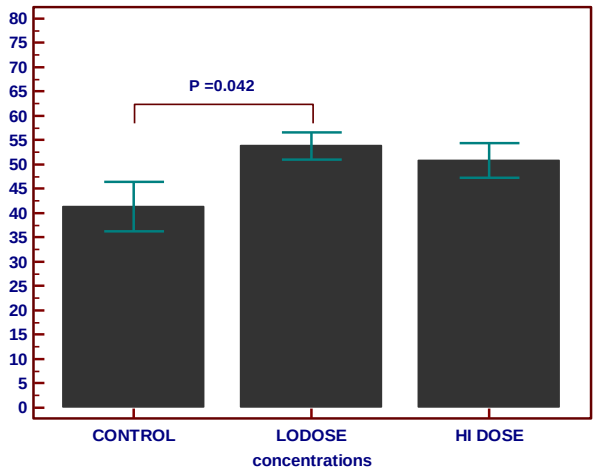

Figure 1: Comparison of the percentage of sperm motility within the "control" (group of rats not treated with *Artemisia Herba-Alba* extract), "low dose" (group of rats force fed with 10mg/kg *Artemisia Herba-Alba* extract) and "h igh dose" (group of rats force fed with 100 mg/kg *Artemisia Herba-Alba* extract). Motility was significantly increased (P=0.042) after treatment with the low dose of *Artemisia Herba-Alba* whereas the high dose compared to the control showed an increase that did not reach a level of significance (P=0.5737).

1.2 Sperm morphology

The effect of an aqueous extraction of *Artemisia Herba-Alba* on sperm morphology is illustrated in figure 9. Treatment with the low dose (10mg/kg) *Artemisia Herba-Alba* extract showed to have had a decrease on the percentage of sperm with normal morphology (96.60±1.05%). Although the mean was lower to the control (97.05±1.12%), the level of significance (P=0.3653) was not reached. However, a significant decrease (P<0.01) in the percentage of sperm with normal morphology was observed after treatment with the high dose (93.8±1.16%) (100mg/kg) of *Artemisia Herba-Alba* extract when compared to the control‖ (97.05±1.12%) (Figure 2)

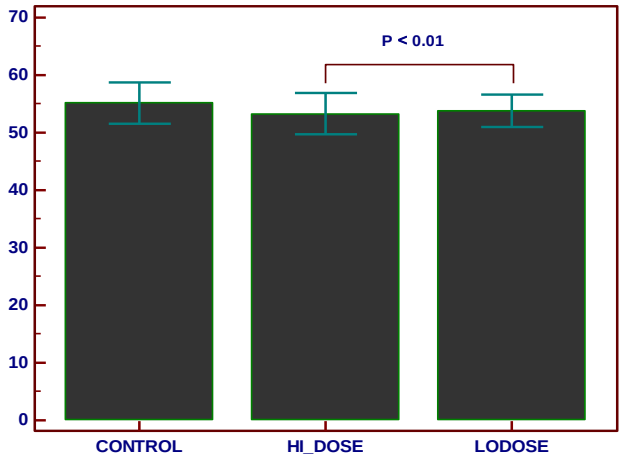

Figure 2: Effect of treatment with *Artemisia Herba-Alba* extract on normal sperm morphology within the "control" (group of rats not treated with *Artemisia Herba-Alba* extract), "low

dose" (group of rats force fed with 10mg/kg *Artemisia Herba-Alba* extract) and "high dose" (group of rats force fed with 100 mg/kg *Artemisia Herba-Alba* extract). The percentage of sperm with normal morphology in the low dose group showed a decrease when compared to the control. However, this decrease was not significant (P=0.3653). An extremely high significant decrease (P<0.01) in the percentage of sperm with normal morphology was detected in the high dose group compared to the control.

1.3 Sperm with intact Δψm

The effect of *Artemisia Herba-Alba* extract on rat sperm with intact Δψm. The graph shows that treatment with the low dose (10mg/kg) *Artemisia Herba-Alba* extract caused an increase in the percentage of sperm with intact Δψm (61.19±8.67%). Although higher than the control (50.33±14.42%), the difference showed to be only marginally significant (P=0.052). Furthermore, treatment with *Artemisia Herba-Alba* extract in the high dose (100mg/kg) also had an increasing effect on the percentage of sperm with intact Δψm (56.85±5.41%). However, the difference to the control (50.33±14.42%) was not significant (P=0.1973). Also of notable importance, the effect of treatment with *Artemisia Herba-Alba* extract on sperm with intact Δψm showed a decrease, as dosage of the extract was increased from 10mg/kg (61.19±8.67%) to 100mg/kg (50.33±14.42%) (Figure 3).

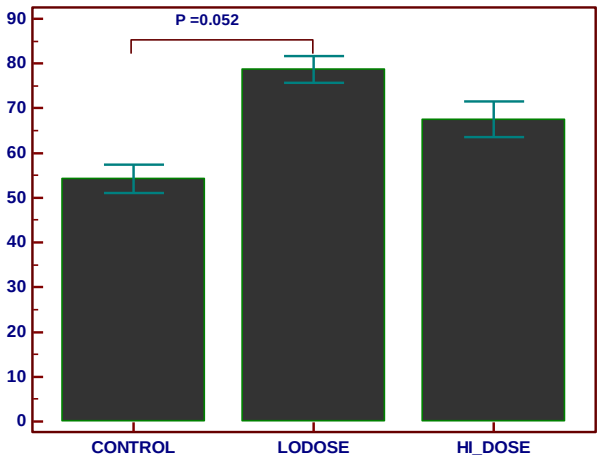

Figure 3: Effect of treatment with *Artemisia Herba-Alba* extract on the percentage of sperm with intact mitochondrial membrane potential (Δψm) within the "control" (group of rats not treated with *Artemisia Herba-Alba* extract), "low dose" (group of rats force fed with 10mg/kg *Artemisia Herba-Alba* extract) and "high dose" (group of rats force fed with 100 mg/kg *Artemisia Herba-Alba* extract). The percentage of sperm with intact Δψm showed an increase after treatment with the low dose when compared to the control, this increased reached a marginal significance (P=0.052). The high dose group also showed an increase for this parameter, however this increase did not reach significance (P=0.1973).

1.4 TUNEL-positive sperm

The percentage of TUNEL-positive sperm represents the percentage of sperm with DNA fragmentation/damage which was detected by means of the TUNEL assay. Treatment with the low dose (10mg/kg) of *Artemisia Herba-Alba* extract had a positive effect on the percentage of DNA fragmented sperm (30.07±3.47%). Although lower than the control (32.74±5.24%), this decrease was not significantly different (P=0.1960). On the other hand, the effect of treatment with *Artemisia Herba-Alba* extract in the high dose (100mg/kg) caused an increase in the percentage of sperm with DNA fragmentation (36.15±6.17%). Although this increase was higher when compared to the control (32.74±5.24%), the difference, however, was significant (P=0.028). In addition, an increase in the percentage of TUNEL-positive sperm was observed after rats were treated with the high dose (100mg/kg) instead of the low dose (10mg/kg) *Artemisia Herba-Alba* extract (Figure 4).

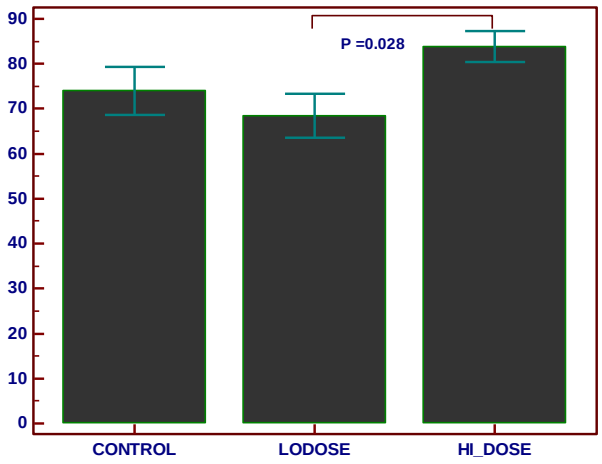

Figure 4: Effect of treatment with *Artemisia Herba-Alba* extract on the percentage of TUNEL-positive sperm within the "control" (group of rats not treated with *Artemisia Herba-Alba* extract), "low dose" (group of rats force fed with 10mg/kg *Artemisia Herba-Alba* extract) and "high dose" (group of rats force fed with 100 mg/kg *Artemisia Herba-Alba* extract). Treatment with the low dose of *Artemisia Herba-Alba* extract showed a decreasing effect on the percentage of TUNEL-positive sperm whereas the high dose group showed an increase (P=0.028) in the percentage of fragmented sperm.

1.5 ROS production

The effect of the *Artemisia Herba-Alba* extract on sperm ROS production is illustrated in figure. Treatment with the low dose (10mg/kg) of the extract had a decreasing effect on the percentage of ROS-positive sperm (40.99±8.53%). Yet, this decrease was not significantly (P=0.1112) lower when compared to the control (50.35±15.48%). Sperm ROS production increased after treatment with the high dose (100mg/kg) of *Artemisia Herba-Alba* extract (51.16±10.33%). Nevertheless, this slight increase showed significance (P=0.022) when compared to the control (50.35±15.48%). Also, an increase in sperm ROS production was observed after treatment with the high dose‖ (100mg/kg) of *Artemisia Herba-Alba* extract when compared to that within the low dose group (10mg/kg). (Figure 5).

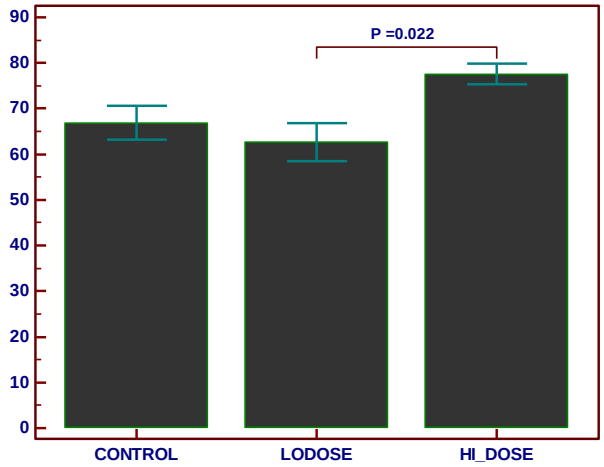

Figure 5: Effect of treatment with *Artemisia Herba-Alba* on the percentage of sperm ROS production within the: "control" (group of rats not treated with *Artemisia Herba-Alba* extract), "low dose" (group of rats force fed with 10mg/kg *Artemisia Herba-Alba* extract) and "high dose" (group of rats force fed with 100 mg/kg *Artemisia Herba-Alba* extract). The percentage of ROS-positive sperm was although not significant (P=0.1112), decreased after treatment with the low dose of *Artemisia Herba-Alba* whereas treatment with the high dose‖ showed to have significant (P=0.022) increasing effect on this parameter.

1.6 Testosterone concentration

Demonstrates the effect of *Artemisia Herba-Alba* extracts on serum testosterone concentration. After treatment with *Artemisia Herba-Alba* extract in the ―low dose‖ (10mg/kg) (3.17±1.83ng/ml) a significant increase (P=0.026) was observed in the concentration of serum testosterone (ng/ml) when compared to the control (1.28±121ng/ml). Also, the effect of *Artemisia Herba-Alba* extract in the high dose (100mg/kg) (4.02±3.30ng/ml) caused a significant increase (P=0.016) in serum testosterone concentration when compared to that within the control (1.28±121ng/ml). An increase, although not significant (P=0.4820) in the concentration serum testosterone was detected after rats were fed with the high dose (100mg/kg) as appose to the low dose (10mg/kg) *Artemisia Herba-Alba* extract (Figure 6).

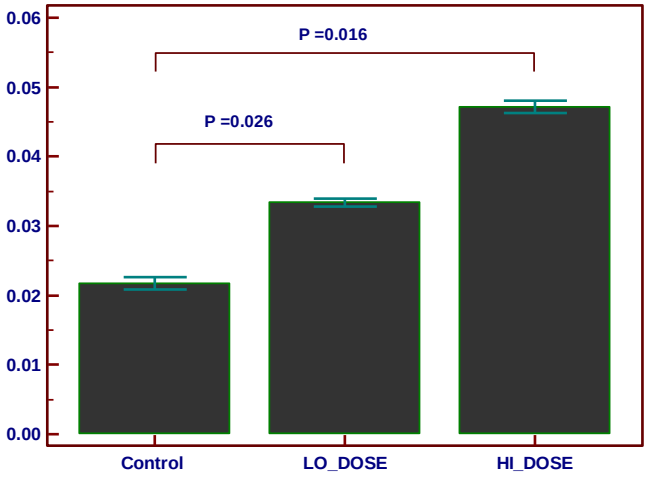

Figure 6: Effect of treatment with *Artemisia Herba-Alba* extract on serum testosterone concentration (ng/ml) within the "control" (group of rats not treated with *Typha capensis* extract), "low dose" (group of rats force fed with 10mg/kg *Artemisia Herba-Alba* extract) and "high dose" (group of rats force fed with 100 mg/kg *Artemisia Herba-Alba* extract). Significant increases were detected after treatment with both the low dose (P=0.026) and high dose (P=0.016) of *Artemisia Herba-Alba* extract when these groups were compared to the control.

Discussion

Effects of an aqueous extraction of *Artemisia Herba-Alba* on different parameters investigated within this particular study

1. Sperm motility.

According to [22]. the percentage of motile spermatozoa within a sample could be predictive of successful conception and therefore was significantly and positively correlated with the fertilizing capacity of spermatozoa *in vitro* [23,24]. In this regard, the importance of this parameter can therefore not be overemphasized. Results of the present study reveal that the aqueous extraction of *Artemisia Herba-Alba* in the low dose (10mg/kg) showed a significant increasing effect (P=0.0484) on sperm motility when compared to the control.The effect of the traditional remedy on motility is dose dependant as the extract in the high dose (100mg/kg) showed less of an increase reaching a difference which was not significant (P=0.5737) when compared to the control. These results are in contrast to the findings of an *in vitro* study performed by [25]. where 1 µg/ml final concentration of an aqueous *Artemisia Herba-Alba* extract had a significant detrimental effect on sperm function, including motility. This contradiction observed in the current *in vivo* study could have been induced by the effects of metabolism on the extract, the activity of the liver as well as the amount of herbal extract absorbed. Moreover, although zinc is absolutely essential for normal human sperm development, its removal from the outer dense fibres during epididymal maturation of the spermatozoon is a prerequisite for sperm to obtain progressive motility [26]. The leaves of *Artemisia Herba-Alba* are known to be saturated with tannins thus, it can be assumed that the rhizomes also contain heavy metal chelating abilities [27].

2. Production of sperm reactive oxygen species (ROS).

Although the mechanism of how reactive oxygen species (ROS) diminish sperm motility remains indefinable, several authors have correlated increased ROS levels with decreased sperm motility [28,29]. An earlier study by [30]. Illustrated that ROS readily attack the polyunsaturated fatty acids within the sperm‘s plasma membrane causing severe lipid peroxidation. In support of this observation, recent studies showed and therefore corroborated that the loss of motility is highly correlated with lipid peroxidation of the spermatozoa [31,32]. Subsequently, in terms of the aetiology of impaired human sperm function and male infertility, ROS-mediated damage has been found to be a significant contributing factor [33].

However, the human body has developed different defense mechanisms to protect itself against the devastating effects caused by ROS-mediated damage. It is known that antioxidants like vitamin E, vitamin C [34]. and coenzyme Q-10 [35]. protect spermatozoa against peroxidative damage. not only observed the peroxidase protective abilities of coenzyme Q-10, they also demonstrated that this particular non-enzymatic antioxidant enhances motility[36].

3. Sperm mitochondrial membrane potential (Δψm).

These results are substantiated by previous works published confirming that a positive relationship does exist between motility and the percentage of sperm with intact Δψm [37,38,39]. A similar positive association was observed between these two parameters, after treatment with *Artemisia Herba-Alba* extract in the high dose (100mg/kg) caused an increase in the percentage of sperm with intact Δψm, although no significance was reached (P=0.1973). The dose dependant effect of *Artemisia Herba-Alba* is once again observed when a decrease in the percentage of sperm with intact Δψm is caused by the high dose (100mg/kg) when compared to that in the low dose (10mg/kg). Treatment of the rats with the low dose (10mg/kg) of *Artemisia Herba-Alba* extract caused a decrease in the percentage of sperm producing an excessive amount of ROS. Although this difference, when compared to the control, showed no significance (P=0.1112), according to [40]. less ROS production by spermatozoa would lead to a decrease in sperm DNA fragmentation. Important to note that a fine balance in the concentration of ROS needs to be maintained, as the presence of minute amounts of ROS is crucial for cell survival and only the excessive production of ROS becomes detrimental to spermatozoa [41]. Moreover, the effect of ROS has shown to be concentration dependant, meaning that at appropriate levels these effects induced by ROS could actually become beneficial to the cell [42]. The decreasing effect that *Artemisia Herba-Alba* had on sperm ROS production could have been caused by the anti-oxidative capacity of both tannins and flavanoids present within the extract.

4. Sperm DNA fragmentation.

The percentage of TUNEL-positive sperm representing the percentage of sperm with fragmented DNA was decreased by the low dose (10mg/kg) of *Artemisia Herba-Alba* extract when compared to the control. Although the decrease in sperm DNA fragmentation was not significant (P=0.1960) (Figure 4), less DNA fragmentation

occurring within sperm nuclei would lead to better sperm functionality and ultimately result in a better fertilization rate. Results also show that treatment with the high dose (100mg/kg) of *Artemisia Herba-Alba* extract caused an increase in DNA fragmented sperm when compared to that within the control‖. Again, although this difference was not significant (P=0.1992), an increase in strand breaks could not have been beneficial to the nuclear DNA within the sperm head. Furthermore, the aqueous extraction of *Artemisia Herba-Alba* in the high dose (100mg/kg) had a negative effect on the percentage of TUNEL-positive sperm when compared to the effect of the low dose (10mg/kg). Also, these results confirm that indeed ROS production can cause DNA fragmentation in sperm [44]. seeing that both the percentage of sperm ROS production and the percentage of TUNEL-positive sperm showed to have increased after treatment with the high dose. Results from the control show significant negative correlations between the percentage of TUNEL-positive sperm and motility (r=-0.774; P=0.0087) as well as between the percentage of DNA fragmented sperm and the percentage of sperm with intact $\Delta\psi m$ (r=-0.817; P=-0.0039). The strong negative correlations were duly expected as they confirm the positive relationship that does exist between motility and sperm with intact $\Delta\psi m$. Furthermore, within the control, results show a significant positive correlation (r=0.681; P=0.0303) between the percentage of sperm with DNA fragmentation and the percentage of ROS-positive sperm. In this regard, previous literature supports the concept that ROS production leads to DNA fragmentation, hence a positive relationship between these two parameters was expected [43].

5. Normal sperm morphology.

Interestingly, the percentage of sperm with normal morphology was lower in the group fed with the low dose (10mg/kg) *Artemisia Herba-Alba* when compared to the control. Even though this decrease did not reach significance level (P=0.3653), several authors have remarked that a decrease in normal sperm morphology would certainly lead to a decrease in motile spermatozoa [44,45]. However, a significant decrease (P<0.001) in the percentage of sperm with normal morphology was observed when the dose of *Artemisia Herba-Alba* extract was increased to 100mg/kg per body weight. Treatment with *Artemisia Herba-Alba* extract in the high dose‖ (100mg/kg) was much more detrimental to the percentage of sperm with normal morphology than the extract fed in the low dose (10mg/kg). These results are in contrast to the positive effect of *Artemisia Herba-Alba* extract observed on sperm motility and the percentage of sperm with intact $\Delta\psi m$.

6. Concentration of serum testosterone.

Testosterone is the male hormone secreted by the Leydig cells located within the interstitium of the testis. Among the other functions of this hormone, it is also responsible for the maintenance of sperm cell production and is known to influence sexual behavior [46].In this study, the concentration of testosterone within serum was determined by means of a Testosterone ELISA. Treatment with *Artemisia Herba-Alba* extract in the ―low dose‖ (10mg/kg) resulted in a significant increase (P=0.026) in the concentration of this androgen present within serum (Figure 6). Furthermore, when the dose of *Artemisia Herba-Alba* extract was increased to 100mg/kg, yet another significant difference (P=0.016) in testosterone concentration was observed when compared to the control. Subsequently, the dose dependant effect of *Artemisia Herba-Alba* extract showed to

cause an even further increase in the concentration of serum testosterone. This increase in serum testosterone concentration may be the reason for the overall beneficial effect of *Artemisia Herba-Alba* extract in the low dose‖ (10mg/kg) on almost all the functional sperm parameters with the exclusion of sperm morphology An increase in sperm function as observed in this regard would result in higher fertilization capacity. In support of this notion, a recent study by [47].documented that lower concentrations of serum testosterone was related to a decrease in sperm quality which in turn would increase a man‘s risk for infertility. These findings regarding the concentration of serum testosterone determining sperm quality is quite conceivable seeing that the formation of sperm cells is testosterone dependant. Therefore, the amount of spermatozoa produced within the testes is also dependant on the concentration testosterone present within serum [48].Hence, compounds found in the extracts of *Artemisia Herba-Alba* might be responsible for enhancing serum concentration which resulted in an increase in sperm quality observed in the low dose group (10mg/kg). It is also possible that the group of phytosteroids found within *Artemisia Herba-Alba* [49].could have been metabolized into androgen-like substances like testosterone, which led to the increased concentration of serum testosterone found within the group of rats treated with the herbal extract. Alternatively, compounds of *Artemisia Herba-Alba* might have acted on the hypothalamus or pituitary resulting in elevated levels of GnRH and luteinizing hormone (LH), respectively. These, in turn, could have acted on the anterior lobe of the pituitary gland and the Leydig cells, respectively, resulting in an increased testosterone production [50.51]. Considering that it was not within the scope of this study to investigate GnRH and LH levels, indeed more work needs to be done in this area in order to ascertain the validity of either of both these hypotheses.

Conclusion : *Artemisia Herba-Alba* truly enhances semen parameters in the rat. In an attempt to confirm and authenticate claims by traditional healers of the plant having the ability to enhance male fertility, one needs to identify all the active compounds that could potentially elicit an effect on the body whether the effect is beneficial or adverse. In this regard, the present *in vivo* study provides information for further phytochemical isolation and characterization studies of active compounds. Further work needs to be done to convincingly elucidate the effects of *Artemisia Herba-Alba* extract on parameters like motility, sperm with intact $\Delta\psi m$, ROS production by spermatozoa, sperm DNA fragmentation, sperm morphology, serum testosterone production.

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تأثير الاستخراج المائي لأرتيميسيا هيربا ـ ألبا على على الوظائف المنوية القياسية في ذكور الجردان

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ملخص البحث

تتمتع أنواع الأرتماسيا بقيمة اقتصادية عالية في العديد من المجالات ، مثل النباتات الغذائية وكمضاد للديدان ومضاد للملاريا في الطب وجدت العديد من أنواع الأرتماسيا طريقها إلى الطب الشعبي. في ليبيا يشار إليه عادة باسم الشيح ، يتم تمثيل جنس Artemisia في ليبيا بسبعة أنواع هي *A. arborescens L* ، و *A. campestris L* ، و *A. glutinosa* ، و *A. herba-alba* ، و *A. judaica* ، و *A. monosperma* ، و *A. variabilis*. وثقت هذه الدراسة ، ولأول مرة ، آثار الاستخلاص المائي لنبات الأرتماسيا *Herba-Alba* في الفئران التي تبحث في وظائف التكاثر الذكرية. من النتائج التي تم الحصول عليها ، يتضح أن المستخلص العشبي في "جرعة منخفضة" (10 مجم / كجم) كان له تأثير مفيد على جميع المعلمات ، مع استبعاد مورفولوجيا الحيوانات المنوية. في هذا الصدد ، قد تسببت المركبات النشطة الموجودة في النبات في زيادة إنتاج هرمون التستوستيرون في الدم مما أدى إلى تحسين جودة الحيوانات المنوية في الفئران. تفسير آخر لزيادة وظائف الحيوانات المنوية ربما يكون ناتجًا عن وجود مكونات داخل نبات لأرتيميسيا هيربا ـ ألبا مما تسبب في الإزالة الفعالة للزنك من الحيوانات المنوية مما يتيح لها الوصول إلى الحركة المثلى. من الممكن أيضًا أن يكون هذان التفسيران المذكوران أعلاه قد حدثا في وقت واحد ، وبالتالي تعزيز الآثار الإيجابية التي لوحظت في هذه الدراسة. أشارت هذه الدراسة إلى أن الاستخدام التقليدي لهذه الأعشاب له آثار على الصحة الإنجابية للذكور.

الكلمات المفتاحية: *الأرتماسيا هيربا ألبا* ,حركية الحيوانات المنوية, تشكل مورفولوجيا الحيوانات المنوية, أنواع الاكسجين التفاعلية للحيوانات المنوية, جهد غشاء الميتوكوندريا (ميكرومتر), تجزئة الحمض النووي, تركيز التستوستيرون.